REMARKS

Objections to the Disclosure

The disclosure is objected to because it contains browser executable codes. The Examiner states that all URLs should be removed from the specification.

Applicants have amended the specification to remove all references to URLs.

Rejection of Claims 46-72 Under 35 U.S.C. §112, First Paragraph

Claims 46-62 are rejected under 35 U.S.C. §112, first paragraph, as it is stated that the specification, while being enabling for the nucleic acid encoding the Zebrafish ferroportin1 transporter (SEQ ID NO: 1), does not reasonably provide enablement for the nucleic acid(s) encoding the human polypeptide SEQ ID NO: 6. Claims 53 and 64 have been canceled.

35 U.S.C. §112, first paragraph requires that the specification enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention of the claims commensurate in scope with these claims.

The Examiner discusses the utility of the polypeptide or protein referred to in some of the claims in question. The issue to be considered, as stated initially in the rejection, is whether the specification provides an enabling description that will allow a person skilled in the art to make and use the invention of Claims 46-72. These claims are drawn to isolated nucleic acids, nucleic acid vectors, cultured cells, and methods for producing a polypeptide. The claims are not drawn to polypeptides.

The invention of Claims 46-72 is fully enabled. The isolation of the ferrportin1 genes of zebrafish, mouse and human is described in Example 2 of the specification on page 57, lines 1-28. The nucleotide sequences are fully described in the Sequence Listing. Using this information, and methods known in the art, one of ordinary skill in the art would know how to make the invention.

One of ordinary skill in the art would know how to use the isolated nucleic acids, nucleic acid vectors, and cultured cells of the claims, and could carry out methods for producing a polypeptide, as in Claims 70 and 71, all without specific instructions, using methods available in the prior art. It is not necessary that an isolated nucleic acid be capable of being incorporated

into an expression system that can produce a polypeptide of a specific type of function or a specific level of function, for the isolated nucleic acid to be capable of use by one of ordinary skill in the art. There are many uses of nucleic acids, and of nucleic acid vectors and cultured cells incorporating those nucleic acids, that do not require the expression of a functional protein, or the expression of any polypeptide at all. The specification at page 45, line 21 to page 50, line 27 provides examples of how one of ordinary skill in the art could use the nucleic acids of the claims. The vectors and cultured cells of the claims can be used to propagate the nucleic acids.

Work published after the filing date of the application shows that the human ferroportin1 gene described in the specification encodes a protein with iron transport function. See Montosi, G. *et al.,* J. Clin. Invest. 108:619-623, 2001; copy enclosed as Exhibit A.

Claims 47, 54, 57, 60, 62 and 67 are rejected under 35 U.S.C. §112, as it is stated that the specification is not enabling for various forms of the polypeptide encoded by SEQ ID NO: 5 and 7, wherein the DNA sequence is at least about 80% identical to the nucleic acid sequences encoding SEQ ID NO: 6. The Examiner states that although the specification discloses the human transporter encoded by SEQ ID NO: 5 and 7, "there is no discussion or working examples disclosed in the instant case, as to what amino acids are necessary to impart or maintain the functional characteristics of the claimed polynucleotide(s)." The Examiner further states that families of transporter proteins can have members with structural similarities but disparate functions. The Examiner states that it is therefore not predictable as to which amino acids are necessary to predict the functional characteristics of the protein.

The Examiner is assuming that the claims in question, drawn to isolated nucleic acids, nucleic acid vectors, and cultured cells, contain a requirement for the nucleic acid, or the nucleic acid within the vector or cultured cell, to encode a protein that functions in a specific way. Such a requirement is not stated in Claims 47, 54, 57, 60, 62 and 67, nor is it implicit in these claims. The sequences referred to in the claims are to be found in the Sequence Listing. The variations on the sequences set forth in the claims are easily understood by one of ordinary skill in the art, and the nucleic acids, vectors and cultured cells of the claims in question can be easily made by one of ordinary skill in the art.

Claims 49, 50, 58, 63, 64 and 68 are rejected under 35 U.S.C. §112, first paragraph, as it is stated that the specification is not enabling for the fragments of the polypeptide encoded by

SEQ ID NO: 5 and 7. The Examiner states that the claims read on both defined and undefined fragments of the polynucleotides encoding SEQ ID NO: 6. However, neither the specific activities of the proteins nor assays to measure these activities are disclosed. The Examiner further states, "There is no discussion or working examples, disclosed in the instant case, as to what amino acids are necessary to maintain the functional characteristics of the polypeptide fragments encoded by the claimed polynucleotides."

The Examiner seeks an activity for polypeptide fragments encoded by the isolated nucleic acids, vectors and cultured cells of the claims. It is not necessary for Applicants to describe such an activity, as polypeptide fragments are not the subject of Claims 49, 50, 58, 63, 64 and 68. What is required is that one of ordinary skill in the art be able to make and use the invention of the claims. Making the invention should present no difficulty to one of ordinary skill in the art, as the sequences referred to in the claims are in the Sequence Listing, and one of ordinary skill could easily produce nucleic acids, vectors and cells as described in the claims. The nucleic acids of the claims, and the vectors and cells comprising such nucleic acids, do not need to encode a full-length protein or a polypeptide of some prescribed activity, to have a reasonable use to one of ordinary skill in the art. Nucleic acids of a length substantially less than SEQ ID NO: 5 or SEQ ID NO: 7 have uses to one of ordinary skill in the art. Some of these uses are described at page 45, line 21 to page 50, line 27 of the specification.

Claim 51 is rejected under 35 U.S.C. §112, first paragraph, as it is stated that the specification is not enabling for the polypeptide or polynucleotide allelic variant recited therein. The Examiner states that neither allelic variants of the Ferroportin1 protein recited in the claim nor allelic variants of the polypeptide encoded by SEQ ID NO: 6 have been identified, and the gene has not been identified to a particular locus. In addition, the Examiner states that Claim 51 "encompasses numerous undefined variants of SEQ ID NO: 5, without precise recitations of function that can be applied to allelic variants." The Examiner contends that it is not clear as to how much variation in the protein may exist while still maintaining the functional characteristics of the protein.

The Examiner is imposing requirements that are not necessary for enablement of the invention of the claim. It is not relevant that the gene has not been identified to a particular locus. Making and using the nucleic acids of the claims does not require one of ordinary skill in

the art to know how much variation can exist in the protein while still maintaining the functional characteristics of the protein. One of ordinary skill in the art would only need to know how to identify polymorphisms in the ferroportin1 gene. A number of methods to identify various types of polymorphisms are known in the art, using the DNA sequences provided in the specification.

Rejection of Claims 55, 58, 64 and 68 Under 35 U.S.C. §102(b)

Claims 55, 58, 64 and 68 are rejected under 35 U.S.C. §102(b) as being anticipated by Fujiwara *et al.* (1995). The Examiner states that the claims read on a "polynucleotide 'portion,'" which can be any length," so that the Fujiwara *et al.* clone falls within the scope of Applicants' claimed invention. Claim 64 has been canceled.

The Fujiwara reference discloses only a DNA sequence of a segment of DNA. The Fujiwara reference does not disclose all elements of the rejected claims. Claim 55 is drawn to an isolated nucleic acid encoding a fusion polypeptide. The Fujiwara reference does not describe an isolated nucleic acid encoding a fusion polypeptide. Claim 58 is drawn to a nucleic acid vector. The Fujiwara reference does not describe a nucleic acid vector. Claim 68 is drawn to a cultured cell. The Fujiwara reference does not describe a cultured cell.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned attorney at (978) 341-0036.

Respectfully submitted,

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MARKED UP VERSION OF AMENDMENTS

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Replace the paragraph at page 8, line 16 through page 9, line 6 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

The nucleic acid molecules of the invention can comprise, in addition to sequences identified by SEQ ID NO or sequences related to these by variations and by hybridization as described herein, other sequences encoding unrelated (heterologous -- that is, with insignificant sequence similarity to a [Ferroprotin1] Ferroportin1) polypeptides or peptides. These peptides or polypeptides can be whole proteins, as occur naturally or as have been modified by design. Together, the nucleic acid sequences make up genes for hybrid or fusion proteins. For example, an unrelated marker sequence that facilitates purification (e.g., by affinity column) of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence can be a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al., Proc. Natl. Acad. Sci. USA 86*: 821-824 (1989), or an HA tag (Wilson *et al., Cell 37*: 767 (1984)), or a sequence encoding glutathione S-transferase of *Schistosoma japonicum* (vectors available from Pharmacia; see Smith, D.B. and Johnson K.S., *Gene 67*:31 (1988) and Kaelin, W.G. *et al., Cell 70*:351 (1992)). For additional applications, the unrelated nucleic acid sequence can encode a peptide or polypeptide which is immunogenic or which enhances the immunogenicity of the fusion protein or polypeptide. Nucleic acids of the invention also include, but are not limited to, nucleic acids comprising a structural gene and its naturally associated sequences that control gene expression.

Replace the paragraph at page 20, line 8 through page 21, line 17 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

The comparison of sequences and determination of percent similarity between two sequences can be accomplished using a mathematical algorithm. (*Computational Molecular Biology*, Lesk,

[A.M.,ed.,] A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects,* Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part 1,* Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology,* von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer,* Gribskov, M. and Devereaux, J., eds., M. Stockton Press, New York, 1991). In a preferred embodiment, the percent similarity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, WI [(see http://www.gcg.com)], using, for example, a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent similarity between two nucleotide sequences is determined using the GAP program in the Wisconsin Package (Devereux, J., *et al., Nucleic Acids Res. 12(1)*:387 (1984)) [(available at http://www.gcg.com)], using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent similarity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (*CABIOS, 4*:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

Replace the paragraph at page 21, line 18 through page 22, line 6 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

The nucleic acids and protein sequences of the present invention can further be used as a "query sequence" to perform a search against databases to, for example, identify other family members or related sequences. Such searches can be performed using the BLASTN, BLASTP, BLASTX, TBLASTN, TBLASTX programs (version 2.0) or PSI-BLAST 2.1 programs based on Altschul, *et al.* (*J. Mol. Biol. 215*:403-10 (1990)). BLAST nucleotide searches can be performed with the BLASTN program, for example, with default parameters matrix = BIOSUM62, gap existence cost = 11, per residue gap cost = 1, lambda ratio = 0.85, filtered, to obtain nucleotide

sequences homologous to (with calculatably significant similarity to) the nucleic acid molecules of the invention. BLAST protein searches can be performed with the BLASTP program, for example, with default parameters scoring matrix = BIOSUM62, word size = 3, E value = 10, gap costs = 11,1 and alignments = 50, to obtain amino acid sequences homologous to the proteins of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al., (Nucleic Acids Res. 25*(17):3389-3402 (1997)). When utilizing BLAST and gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. [See http://www.ncbi.nlm.nih.gov/ BLAST/.]

Replace the paragraph at page 30, line 26 through page 31, line 12 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Isolated Ferroportin1 protein or, an antigenically similar portion thereof, especially a portion that is soluble (e.g., a peptide or a fusion polypeptide comprising at least 10 contiguous amino acid residues of a Ferroportin1), can be used in a method to select and identify molecules which bind specifically to the Ferroportin1. Fusion proteins comprising all of, or a portion of, the [Ferroprotin1] Ferroportin1 linked to a second moiety not occurring in the Ferroportin1 as found in nature, can be prepared for use in another embodiment of the method. Suitable fusion proteins for this purpose include those in which the second moiety comprises an affinity ligand (e.g., an enzyme, antigen, epitope). Ferroportin1 fusion proteins can be produced by the insertion of a gene encoding the Ferroportin1 or a variant thereof, or a suitable portion of such gene into a suitable expression vector which encodes an affinity ligand (e.g., pGEX-4T-2 and pET-15b, encoding glutathione S-transferase and His-Tag affinity ligands, respectively). The expression vector can be introduced into a suitable host cell for expression. Host cells are lysed and the lysate, containing fusion protein, can be bound to a suitable affinity matrix by contacting the lysate with an affinity matrix.

Replace the paragraph at page 40, lines 6 through 20 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Another embodiment of the invention is a method for inhibiting iron export in [Ferroprotin1-expressing] Ferroportin1-expressing cells of a mammal (e.g., a human), comprising administering to the mammal a therapeutically effective amount of an inhibitor of the transport function of Ferroportin1, thereby decreasing iron in the circulation. Hemochromatosis can be due to the inheritance of a mutant gene or due to secondary iron overload from an iron-loading anemia such as thalassemia or sideroblastic anemia. Where it is desirable to reduce the uptake of iron into the circulatory system through the intestine, for example, in the treatment of hemochromatosis in a human, one or more inhibitors of Ferroportin1 can be administered in an effective dose, and by an effective route, for example, orally, or by an indwelling device that can deliver doses to the small intestine. The inhibitor can be one identified by methods described herein, or can be one that is, for instance, structurally related to an inhibitor identified by methods described herein (e.g., having chemical adducts to better stabilize or solubilize the inhibitor). The invention further relates to compositions comprising inhibitors of iron uptake in a mammal, which may further comprise pharmaceutical carriers suitable for administration to a subject mammal, such as sterile solubilizing or emulsifying agents.

Replace the subtitle at page 58, line 6 with the below subtitle marked up by way of bracketing and underlining to show the changes relative to the previous version of the subtitle.

Example 3: Expression of [*ferroprotin1*] *ferroportin1*